increase in enzymes responsible for ammonia production favours the view that increased urinary ammonia is caused, at least in part, by an increased formation of ammonia.

SUMMARY

1. Changes in ammonia excretion were produced in rats by the induction of chronic acidosis and chronic alkalosis. After 3–8 months, some selected renal enzymes were assayed in these animals by determining ammonia production, by kidney slices, from L-glutamine, glycine, L-leucine, L-aspartic acid and L-alanine. These substrates were selected to investigate possible adaptation of four enzymes which might be concerned in ammonia production: L-glutaminase, glycine oxidase, L-amino-acid oxidase and the transaminase system.

2. Chronic acidosis resulted in an appreciable increase, and chronic alkalosis in an appreciable decrease, in ammonia production from L-glutamine, glycine and L-leucine. There was no significant change in ammonia production from L-aspartic acid and L-alanine.

3. These results suggest that urinary ammonia is produced, at least in part, by (1) deamination of glutamine, (2) deamination of glycine by glycine oxidase, (3) deamination of other monoamino monocarboxylic acids by L-amino-acid oxidase.

4. Reasons are given for supposing that deamination of L-alanine is effected chiefly, if not entirely, by the transaminase system which also acts upon L-aspartic acid. It would then appear either that this system plays no part in the normal mechanism of urinary ammonia formation, or that one or more of its component enzymes is not adaptive.

5. The adaptive changes found in three renal enzymes indicate that acidosis and alkalosis cause a change in ammonia formation and not merely in ammonia elimination.

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Kinetic Studies of the Metabolism of Foreign Organic Compounds

2. THE FORMATION OF PHENOLS FROM CERTAIN PRECURSORS

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The introduction of a hydroxyl group into the benzene nucleus is recognized as a common metabolic process. Compounds which are hydroxylated in this way may be regarded as phenol precursors. The present investigation deals with phenol precursors which are hydroxylated to give predominantly one isomer, namely anisole, which gives *p*-methoxyphenol (Bray, Thorpe & Wasdell, 1951*a*), *N*-phenylurea, which gives *N*-(*p*-hydroxyphenyl)-urea (Bray, Lake & Thorpe, 1949) and benzoxazolone, which is excreted as a hydroxybenzoxazolone, the configuration of which has not yet been established (Bray, Clowes & Thorpe, 1952*a*). The overall plan was as described previously (Bray, Thorpe & White, 1951*b*). In some experiments the metabolites were estimated as phenols, in the

remainder as ethereal sulphate and glucuronide. The results obtained suggest that the rate of formation of phenols from these compounds is proportional to the body level of the precursor.

METHODS

Animals, diet, dosage and collection of urine samples

The animals were maintained on the standard pellet diet previously described (Bray, Ryman & Thorpe, 1947). The compounds (usually 0·7 g.) were administered by stomach tube as suspensions in water, either alone or, more often, with Na_2SO_3 or L-cystine. Administration of these 'sulphate precursors' is usually necessary to ensure that the ethereal sulphate conjugation follows first-order reaction kinetics. This is discussed in detail in a subsequent paper (Bray, Humphris, Thorpe, White & Wood, 1952*c*). The collection of urine samples was as previously described (Bray *et al.* 1951*b*). The water intake was considerable.

Estimation of metabolites

Free and total phenols. A modification of the Folin & Ciocalteu method described by Bray, Thorpe & White (1950*b*), was used with a Spekker photoelectric absorptiometer with Chance filter OR2. The quantities suitable for estimation varied with the phenol but were of the order of 0·1 mg. The slope of the linear calibration curve was of the order of 0·025 mg./0·1 drum reading.

Glucuronic acid. The method was a modification of that described by Hanson, Mills & Williams (1944), the chief alteration being the replacement of amyl alcohol by *n*-butanol, thus obtaining a monophasic system so that separation before reading is unnecessary. This greatly facilitates the analysis of a large number of urine samples.

Reagents. Naphthoresorcinol solution, 0·375% (w/v) in water, was 'aged' by keeping either at room temperature for 2 days or at 0° for 1 week before use. The 'aged' solution can be kept at 0° for 1 week.

Undiluted (approx. 10N) technical grade hydrochloric acid (British Drug Houses Ltd.) was used. The blue colour may not develop if pure HCl is used. The *n*-butanol was British Drug Houses Ltd. ordinary quality. The D-glucurone solution contained 0·1 mg./ml. in water and if kept at 0° was stable for 1 week.

Procedure. The urine was diluted so as to contain the equivalent of not more than 0·04 mg./ml. glucuronic acid. Diluted urine (2 ml.) was placed in a glass tube (13 × 150 mm.), and HCl (4 ml.) and naphthoresorcinol solution (2 ml.) added. The tube was heated in boiling water for 2 hr., evaporation being minimized by the use of a 'cold finger', and then cooled in cold water. After addition of *n*-butanol (5 ml.) the tube was corked, shaken and then left for 2–3 min. to disperse air bubbles. The mixture did not form two layers, thus eliminating the separation of the coloured organic phase (Hanson *et al.* 1944). The absorption value was read in a Spekker photoelectric absorptiometer within 5 min. of the addition of the butanol. An Ilford Spectrum Orange filter 607 was used. If absorption values were read 30 min. after mixing with butanol results were from 0·001 to 0·003 mg. (3–5%) lower per tube. A calibration curve was constructed using, in place of urine, from 0·2 to 2·0 ml. glucurone solution made up to 2 ml. with water. D-Glucuronic acid, D-glucurone and 3:4-dimethylphenylglucuronide in water or urine have been found to give identical linear calibration curves (drum reading 0·1 ≡ 0·015 mg. glucuronic acid per tube). If the naphthoresorcinol solution is insufficiently 'aged' the calibration curve is not linear above 0·05–0·10 mg. glucuronic acid.

Inorganic and ethereal sulphate. A simplified turbidimetric method was used. The following reagents were required: hydrochloric acid, about 7·1N; sodium hydroxide-potassium sulphate solution, NaOH (2·5N) containing K_2SO_4 (0·128 g./l.) (the sulphate is added to ensure that the absorption calibration curve is linear over the required range); $BaCl_2.2H_2O$, 'Analar'; potassium sulphate solution, K_2SO_4 0·44 g./l.

Procedure. Estimations of inorganic and total sulphate were carried out simultaneously so as to ensure uniformity in conditions. For total sulphate, urine (5 ml. containing 0·1–1·0 mg. SO_3) was heated with HCl (2 ml.) in a tube (16 × 150 mm.) in boiling water for 1 hr., evaporation being reduced by use of a 'cold finger'. To this solution, after cooling, NaOH-K_2SO_4 (5 ml.) was added. For inorganic sulphate, NaOH-K_2SO_4 (5 ml.) and HCl (2 ml.) were mixed in a test tube and, after cooling, urine (5 ml.) was added. The tubes for both inorganic and total sulphate were prepared in duplicate. To one of each $BaCl_2.2H_2O$ (200 mg.) was added and the tube vigorously shaken for 30 sec. The absorption value of each of the four solutions was determined with a Spekker photoelectric absorptiometer using a Chance neutral filter H508. Absorption values were read between 7 and 10 min. after mixing. The $BaSO_4$ suspension was stable up to 20 min. A calibration curve was made by using appropriate dilutions of the K_2SO_4 solution. The calibration curve was linear over the range 0–1·0 mg. SO_3 per tube and identical curves were given by potassium, ammonium and ferrous ammonium sulphates. For estimation of ethereal sulphate in urine this method gave results averaging 92% (range 80–105%) of those obtained by the Folin (1905–6) gravimetric method. The average recovery of inorganic sulphate added to urine was 94% (range 82–110%). The presence of sulphite did not interfere with the determination of inorganic or ethereal sulphate by the turbidimetric method. The method was reliable if the ratio inorganic/ethereal sulphate did not exceed 5·0.

THEORETICAL

Expressions relating the amounts of a phenol precursor administered and the amounts of the corresponding phenol (free and conjugated) formed in a given time after administration of the precursor can be derived as follows:

Assuming that an amount A of the precursor is introduced into the body at zero time and that it is hydroxylated by a process having a first-order velocity constant k_b, the resulting phenol being conjugated by processes having a total first-order velocity constant k_m, then the following differential equations may be formulated:

$$\frac{db}{dt} = -\frac{da}{dt} = k_b a,$$

$$\frac{dm}{dt} = k_m (b - m),$$

where a = amount of precursor at time t, b = total amount of phenol (free and conjugated) at time t, and m = amount of conjugated phenol at time t.

Solution of these equations gives

$$a = Ae^{-k_b t}, \quad (1)$$

$$b - m = \frac{k_b A}{k_m - k_b}(e^{-k_b t} - e^{-k_m t}), \quad (2)$$

$$m = A\left[1 - \left(\frac{k_m e^{-k_b t} - k_b e^{-k_m t}}{k_m - k_b}\right)\right]. \quad (3)$$

The kinetic studies reported in this and succeeding papers suggest that the above equations represent the course of the metabolic reactions which occur when a phenol precursor is administered to the rabbit. It will be shown (Bray *et al.* 1952*b*, *c*) that under the conditions of the present experiments the conjugation processes of phenols with glucuronic and sulphuric acids do, in fact, follow first-order reaction kinetics (with velocity constants k_g and k_s respectively), and that excretion of these conjugates by the kidney is efficient, so that their blood level remains low, and their rate of excretion may be taken as being equal to their rate of formation. In general, the rate of formation of the phenol may be found by applying the above equations to experimental data, namely the value of m at different times. Theoretical calculations show, however, that if the value of k_m is at least five times as great as that of k_b, then the rate of excretion of the conjugates will represent, to a close approximation, the rate of formation of the phenol, so that an excretion curve (cf. Bray *et al.* 1951*b*) may be taken as representing the formation of the phenol and graphical treatment applied to deduce a value for k_b.

RESULTS

Anisole. Values of k_b for anisole were calculated by substitution in equation 3 of the mean value for k_m (1·08 hr.$^{-1}$) found for *p*-methoxyphenol (obtained from $k_g + k_s$, Bray *et al.* 1952*b*, *c*) and of the observed values of t and m for anisole. The necessary assumption involved, namely, that the hydroxylation process follows first-order reaction kinetics, was vindicated by the fact that consistent values of k_b were obtained from several different points in any single experiment. The mean values of k_b found in two experiments were 0·20 and 0·16 hr.$^{-1}$. Since these values are less than one-fifth of that of k_m (1·08 hr.$^{-1}$), graphical methods (usually the 'log' method) were then applied (see Bray *et al.* 1951*b*) to all the anisole experiments. It was shown, by the fit of experimentally determined points on theoretical curves corresponding to first-order velocity constants derived by the graphical methods, that the hydroxylation of anisole follows first-order reaction kinetics, i.e. the rate of the reaction is proportional to the amount of anisole in the body, designated 'body level'. The results for a typical experiment are shown in Fig. 1. The values obtained for k_b are given in Table 1. It should be noted that in the derivation of equation 3 it is assumed that the precursor is introduced into the body at zero time. Since experimentally the compounds are absorbed from the alimentary tract, zero time is not known exactly, but extrapolation of the excretion curve to meet the time axis at t_o, as shown for the theoretical curve in Fig. 1, gave values for zero time leading to consistent values for k_b provided that only experimental points after 2–3 hr. were used for the calculation.

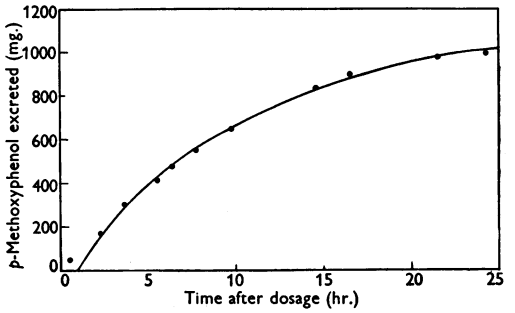

Fig. 1. Excretion of *p*-methoxyphenol by the rabbit after administration of 1 g. anisole. The curve is the theoretical curve for $k_b = 0{\cdot}10$ hr.$^{-1}$ and the points are those obtained by experiment.

Table 1. *Velocity constants (in hr.$^{-1}$) for the hydroxylation of anisole, N-phenylurea and benzoxazolone in the rabbit, determined graphically using the 'log' method*

(Dose 0·7 g. unless stated otherwise.)

Anisole	*N*-Phenylurea	Benzoxazolone
0·10*	0·12	0·21
0·14	0·15	0·37
0·17	0·10	0·21
0·06	—	0·21
—	—	0·29
0·11†	0·12†	0·25†

* Dose 1·0 g.
† Geometric mean, see Bray *et al.* (1952*b*), Table 2.

N-Phenylurea and benzoxazolone. By similar application of equation 3 to data from two experiments with *N*-phenylurea and two with benzoxazolone (for values for k_g and k_s see Bray *et al.* 1952*b*, *c*) the mean values for k_b obtained were 0·20 and 0·21 hr.$^{-1}$ for *N*-phenylurea and 0·35 and 0·37 hr.$^{-1}$ for benzoxazolone. Since the mean values of k_m for the phenols concerned are respectively 0·22 and 0·36 hr.$^{-1}$, the values for k_b derived graphically cannot be taken as true values without further consideration. Values of k_b derived by the

'log' method from excretion curves representing the same experimental data were 0·10 and 0·15 hr.$^{-1}$ for *N*-phenylurea and 0·29 and 0·37 hr.$^{-1}$ for benzoxazolone, i.e. a mean of 0·6 and 0·9 of the mathematically derived values respectively. Since calculation of the mean value of k_b by means of equation 3 involves the solution of the equation by successive approximation for each experimental observation, it is impracticable to treat any considerable number of experiments in this way. The results reported in Table 1 are those derived by the 'log' method as for anisole. It is more likely, however, that the correct mean values of k_b for *N*-phenylurea and benzoxazolone would be $0·12/0·6 = 0·20$ and $0·25/0·9 = 0·28$ hr.$^{-1}$ respectively. The present investigation was primarily intended to supply results necessary for the investigations reported in the next three papers. For this purpose this degree of accuracy is sufficient.

DISCUSSION

Although the values of k_b for the three compounds discussed here are within the range 0·1–0·3 hr.$^{-1}$, it is probable that in general there is a wide range of values for k_b. For example, with compounds which form only traces of hydroxylation products, e.g. *p*-cresol (Bray *et al.* 1950*b*), the value is probably of the order of 0·001–0·01 hr.$^{-1}$. It is possible to calculate an approximate value for k_b for *p*-hydroxybenzamide. This compound, which is hydrolysed to the extent of 10%, has a first-order velocity constant for the hydrolysis of the carbamyl group of 0·05 hr.$^{-1}$ (unpublished result). The extent of hydroxylation is 1–4% of the dose (Bray, Thorpe & White, 1950*a*). Thus k_b for this compound must be about 0·005–0·02 hr.$^{-1}$.

This series of investigations had as its primary stimulus an implication of the generalization (Thorpe, 1950) relating to the metabolism of foreign organic compounds which stated that conjugation appeared to take place more readily than conversion of potential centres for conjugation, and conversion of potential centres more readily than hydroxylation. The implication was that this was a consequence of the values of the velocity constants of the three types of reaction. It was thus expected that the velocity constants of conjugation reactions would be appreciably greater than those of processes for the conversion of potential centres and these, in their turn, greater than the velocity constants of hydroxylation reactions. It can be seen, however, from the examples given in Table 2 that certain values of velocity constants for hydroxylation are comparable in magnitude with those found for conjugation reactions and conversion processes, and that the velocity constants of conjugation processes are not always greater than those of hydroxylation processes.

Table 2. *Mean velocity constants (in hr.$^{-1}$) for conjugation, conversion and hydroxylation reactions in the rabbit*

Conjugations:	
Benzoic acid with glucuronic acid*	0·08
N-*p*-Hydroxyphenylurea with glucuronic acid†	0·11
N-*p*-Hydroxyphenylurea with sulphuric acid‡	0·11
Hydroxybenzoxazolone with glucuronic acid†	0·18
Hydroxybenzoxazolone with sulphuric acid‡	0·18
p-Methoxyphenol with glucuronic acid†	0·54
p-Methoxyphenol with sulphuric acid‡	0·54
Conversions of potential centre for conjugation:	
Toluene to benzoic acid*	0·11
Benzamide to benzoic acid*	0·32
Hydroxylations:	
N-Phenylurea to *N*-*p*-hydroxyphenylurea§	0·20
Benzoxazolone to hydroxybenzoxazolone§	0·28
Anisole to *p*-methoxyphenol§	0·11

* Bray *et al.* (1951*b*). † Bray *et al.* (1952*b*).
‡ Bray *et al.* (1952*c*). § This paper.

SUMMARY

1. An investigation of the kinetics of hydroxylation of anisole, *N*-phenylurea and benzoxazolone in the rabbit has been made.

2. The rates of hydroxylation of these compounds appear to be proportional to their body levels, the velocity constants being 0·11 hr.$^{-1}$ for anisole and approximately 0·20 and 0·28 hr.$^{-1}$ for *N*-phenylurea and benzoxazolone respectively.

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